PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Broyles *et al.*	Group Art Unit: 1632
Serial No.: 10/003,669	Examiner: Janice Li Qian
Filed: November 1, 2001	Atty. Dkt. No.: OMRF:027US/SLH
For: GENE REGULATION THERAPY INVOLVING FERRITIN	

CERTIFICATE OF MAILING
37 C.F.R. § 1.8

I hereby certify that this correspondence is being deposited with the U.S. Postal Service as First Class Mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on the date below:

March 3, 2005 _____
Date Steven L. Highlander

DECLARATION OF DR. ROBERT H. BROYLES UNDER 37 C.F.R. §1.132

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

I, the undersigned, do declare that:

1. I am a citizen of the United States. I am a named inventor on the above-captioned application. I currently hold the position of Professor in the Department of Biochemistry & Molecular Biology at Oklahoma University Health Science Center, and Research Member, in the Free Radical Biology & Aging Research Program at the Oklahoma Medical Research Foundation. A copy of my *curriculum vitae* is attached.

2. In FIG. A (attached), we present a Western blot and an associated bar graph that show induction of the endogenous FtH gene in human cells (NT-2 cells) by abscissic acid added to the medium for 8 days. We have also found this induction of FtH by the same compound in human K562 cells (not shown).

3. In FIG. B (attached), we show initial data from a transgenic mouse made to express human FtH in definitive erythroid cells that are elaborated by the fetal liver beginning in mid-gestation. These red blood cells are the first to express the adult beta-globin genes of the mouse, and the construct in which we inserted the FtH gene was made to direct expression of FtH only in these cells and at the same time as the adult mouse beta-globin. The strain of mouse from which these initial data were collected has ***two*** types of adult beta-globin genes, beta-major (which accounts for 60% of the expression) and beta-minor (40%). The ***key*** to this experiment is that ***only*** the beta-major globin gene of the mouse has the CAGTGC DNA motif that is the core of the FtH binding site; the beta-minor globin gene lacks this site. Thus, when the human FtH transgene is activated in the definitive mouse red cells, the expression of the mouse beta-major globin gene will be repressed but the beta-minor gene will ***not*** be repressed because it lacks the FtH repression site. So, the transgenic mice mice will be expected to be born alive but have a reduced beta-major/beta-minor ratio and a mild beta-thalessemia due to excess alpha-globin chains (because the mouse has no fetal globin gene to up-regulate in place of beta-major). The presence of "target cells" (red blood cells with a dark spot in the center) in the blood smear of these transgenics and the reduced beta-major/beta-minor ratio seeon

the UT-PAGE globin gel constitute evidence that human FtH can function as a beta-globin gene repressor in a living animal.

4. I hereby declare that all statements made herein of my knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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Date Robert H. Broyles, Ph.D.